Current Biology, Vol. 14, R840–R841, October 5, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.09.037

Floral Development: An ABC Gene Chips in Downstream

Dispatch

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In flower development, specification of stamens and carpels requires the *AGAMOUS* gene. A recent study has now shown that *AGAMOUS* also plays more specific roles in the regional activation of sporocyte formation.

The insightful analyses of flower development in *Arabidopsis* and *Antirrhinum* which began in the 1980s helped introduce plant biology to a new era of molecular genetics. These studies led to the formulation, and then confirmation, of the 'ABC' model of floral organ specification [1], now a common feature of biology textbooks. This model predicted that three gene activities — A, B and C — act combinatorially to specify the four floral organ types — sepals, petals, stamens and carpels (Figure 1A). The cloning of the genes representing these activities, and molecular studies made possible by this cloning, validated the ABC model. Most of the genes represented in the ABC model turned out to encode 'MADS box' transcription factors, presumed to act by the combinatorial control of downstream gene expression.

As in related areas of developmental biology — such as the control of segment identity by homeotic genes in *Drosophila* — it has proven difficult to identify the downstream components that translate the organ identity cues provided by the ABC genes into specific effector functions that determine the shaping and cellular differentiation of the floral organs. Recent work by Ito *et al.* [2] goes some way towards bridging this gap by demonstrating that the C activity gene *AGAMOUS* (*AG*), which specifies stamen and carpel development [3], directly activates transcription of *SPOROCYTELESS* (*SPL*), which encodes a member of a novel transcription factor family that promotes sporocyte formation [4].

A different organ type occupies each of the four whorls of the *Arabidopsis* flower, with sepals developing in the first whorl, petals in the second whorl, stamens in the third whorl and carpels in the fourth and innermost whorl. *AG* controls the identity of stamens and carpels, the floral organs that produce the male and female gametes, and is initially expressed throughout these primordia. Later, however, *AG* expression becomes localized to specific regions of these developing organs. Carpel identity only requires *AG* activity, while stamen identity requires the concerted activities of *AG*, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) [1]. Loss-of-function mutations in *AG* result in the conversion of third whorl stamens into petals and replacement of the fourth whorl carpels with a new flower that repeats this same pattern: sepals–petals–petals, sepals–petals–petals, and so on. Ectopic expression of *AG* in the outer whorls can convert petals into stamens and sepals into carpels [5]. While these results clearly show that *AG* is necessary and sufficient for stamen and carpel development, it has been unclear whether *AG* also has later roles in the development of specific regions of these floral organs.

The new work of Ito *et al.* [2] takes our understanding of *AG*'s complex role in organ development a big step forward. The first goal of this study was to identify putative downstream transcriptional targets of *AG* using spotted arrays of cDNAs derived from floral tissue. To facilitate the identification of direct downstream targets, Ito *et al.* [2] made a fusion protein AG–GR, consisting of AG linked to the glucorcorticoid receptor (GR). The GR component normally retains this fusion protein in the cytoplasm, but when its ligand dexamethasone is added to the cell, AG–GR moves into the nucleus, allowing AG to regulate transcription [6]. This allowed Ito *et al.* [2] to regulate AG's activity in a controlled manner and track the transcriptional consequences over time. They found that the *SPL* gene was up-regulated following dexamethasone treatment, confirming this by both PCR and *in situ* hybridization; no sign of *SPL* expression could be detected by *in situ* hybridization in *ag* mutant flowers.

Among the potential *AG* targets, *SPL* must have seemed particularly attractive, as previous work showed that it plays an important role in the development of specialized tissues in *AG*-dependent organs. In stamens, *SPL* is required to promote the differentiation of the primary sporogenous cells known as microsporocytes, as well as cells of the anther wall [4,7]. In wild-type anthers, these tissues produce the pollen grains, the progenitors of the male gametes (Figure 1B). *SPL* also plays a role in carpel development, controlling two aspects of ovule development. One is analogous to its role in promoting the formation of microsporocytes in anthers — *SPL* is required for formation of the megaspore, progenitor of the female gametophyte, which produces the egg cell (Figure 1C). The second is that *SPL* regulates the growth of the integuments, protective sheaths of cells that enclose the female gametophyte in the ovule [7] (Figure 1C).

It was next important to extend this work further to determine whether AG is a direct regulator of *SPL* expression. The DNA-binding activity of MADS box transcription factors has been shown to rely on CArG-box-like sequences in the promoters of target genes [8]. Two imperfect matches to the consensus binding site for AG were found in the *SPL* genomic region. Ito *et al.* [2] tested these sequences using an electrophoretic mobility-shift assay, and found that AG is only able to bind to the distal-most CArG-box-like sequence, and mutation of this sequence abolished this interaction.

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To examine the relevance of these findings *in vivo*, an *SPL* promoter::GUS reporter construct, which included the distal CArG box sequence, was introduced into transgenic plants. The resulting staining pattern mirrored that of *SPL* expression, as detected by *in situ* hybridization. The same mutations that abolished *in vitro* binding of AG to the distal CArG-box were introduced to this construct and found to cause a dramatic reduction in overall staining levels. In stamens, only low levels of staining were detected at the lateral margins, and in ovules, staining in the funiculus (ovule stalk) and outer integuments was completely lost (Figure 1B,C). Importantly, staining of the nucellar tissue in the ovule, a region in which *AG* is not expressed, was unaffected by the CArG box mutation. While the expression domains of *AG* and *SPL* overlap initially, later stages of stamen development are marked by a shift in their expression domains, with *AG* expression being localized to the stamen filament and tapetum during floral stages 8–9, while *SPL* is expressed in the sporogenous cells and tapetum, but not the filament. Ito *et al.* [2] suggest that this evidence is consistent with *AG* being required for the activation of *SPL* expression, but not necessarily for its maintenance.

If *SPL* is necessary for the development of sporogenous tissues in stamens, and this activity is dependent on *AG*, then ectopic expression of *SPL* in an *ag* mutant background should rescue the development of these tissues. Ito *et al.* [2] introduced an SPL–GR construct into an *ag* mutant background and found that induction with dexamethasone resulted in the development of petals with ectopic sporogenous tissues. Interestingly, the development of these ectopic tissues was only observed in the lateral regions of organ primordia and only in whorls interior to the second whorl.

The identification of *SPL* as a direct downstream target of *AG* opens up many new avenues to explore the relationship between the specification of organ identity and the downstream processes that control the differentiation and growth of the floral organs. This work shows that *AG* is important for *SPL* activation, but also suggests that other factors are likely at play that regulate the regional specificity of this activity. While the *AG* expression domain initially encompasses the *SPL* expression domain, *AG* is likely to act with other partners to maintain *SPL* expression in tissues where *AG* expression quickly fades. It will also be interesting to determine the role, if any, that *AP3* and *PI* play in controlling *SPL* expression, as they are also necessary for stamen identity and, like *AG*, are expressed during the later stages of stamen development.

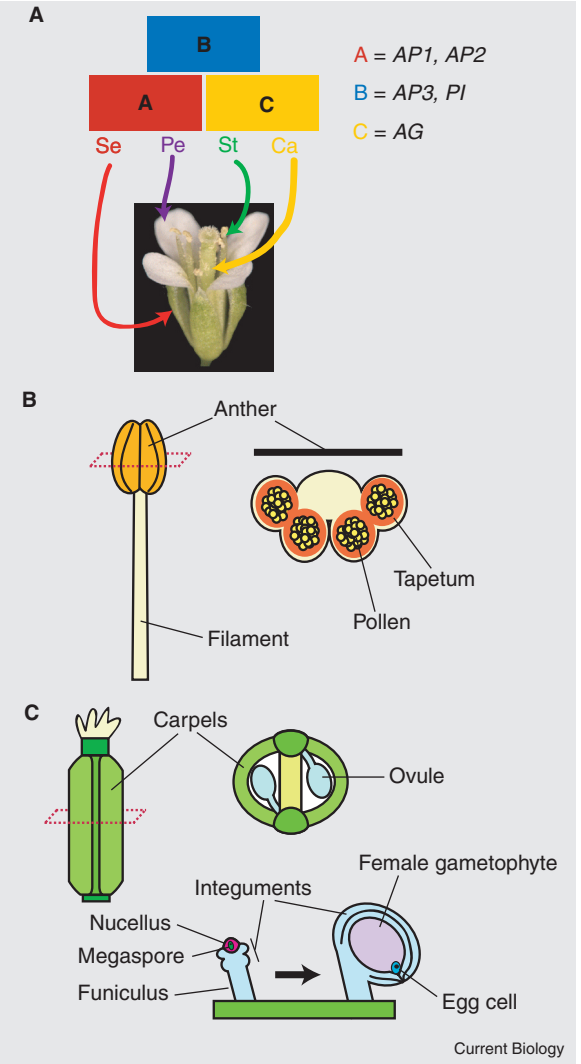

Figure 1. The ABC-model of floral organ development in *Arabidopsis*.

(A) The model proposes that three gene activities represented by the letters A, B and C are involved in determining the identities of the four floral organs. A activity alone specifies sepal identity; A and B activity together specify petal identity; B and C activity specify stamen identity; and C activity alone specifies carpel identity. All genes represented by these activities encode MADS box transcription factors, except *AP2*. Se, sepal; Pe, petal; St, stamen; Ca, carpel. (B) A diagram depicting stamen morphology. (C) A diagram of a gynoecium, the product of two fused carpels, and internal ovules.

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